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The effect of intra-nucleus accumbens administration of allopregnanolone on δ and γ2 $GABA_A$ receptor subunit mRNA expression in the hippocampus and on depressive-like and grooming behaviors in rats

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ABSTRACT

Alterations in $GABA_A$ receptor expression have been associated with the allopregnanolone (3α-hydroxy-5α-pregnan-20-one; 3α,5α-THP) antidepressant-like effect in rats. The present study aimed to verify the effect of bilateral, intra-nucleus accumbens core (intra-AcbC) administration of the neurosteroid allopregnanolone on behaviors in the forced swim and grooming microstructure tests and in the δ and γ2 $GABA_A$ receptor subunit mRNA expression in right and left hippocampus of rats. The results of this study showed that bilateral, intra-AcbC allopregnanolone administration (5 μg/rat) presented antidepressant-like activity in the forced swim test concomitant with an increase in climbing. Allopregnanolone at doses of 1.25 and 5 μg/rat also decreased the percentage of correct transitions in the grooming microstructure test. Both δ and γ2 $GABA_A$ subunit expressions increased in the rat hippocampus after allopregnanolone intra-AcbC treatment. Our findings point to asymmetrical $GABA_A$ receptor expression changes in the hippocampus of animals treated with allopregnanolone. Further investigation should evaluate the antidepressant-like effect of allopregnanolone not only in other directly infused regions but also with respect to changes in other brain areas of the limbic system to understand allopregnanolone's mechanism of action.

1. Introduction

Neuroactive steroids are important endogenous modulators of depressive and anxiety-related behaviors (Schüle et al., 2011). Indeed, allopregnanolone and other neurosteroids, such as dehidroepiandrosterone, pregnenolone (Schüle et al., 2011) and pregnenolone-derivatives (Bianchi and Baulieu, 2012) have been implicated with antidepressant-like effect in rodents. Allopregnanolone (3α-5α-tetrahydroprogesterone; 3α-5α-THP; ALLO), a positive modulator of the $GABA_A$ receptor (Paul and Purdy, 1992), is decreased in the cerebrospinal fluid (Uzunova et al., 1998) and plasma (Romeo et al., 1998; Strohle et al., 1999) of depressive patients. Furthermore, clinically effective treatment with antidepressants is correlated with increased levels of allopregnanolone in the plasma of depressive individuals (Romeo et al., 1998; Strohle et al., 1999).

Likewise, preclinical studies show that allopregnanolone is decreased in specific brain areas of the olfactory bulbectomized rat model of depression compared with sham-operated rats (Uzunova et al., 2003). Moreover, there is an increase in allopregnanolone levels in mouse and rat brain after subchronic antidepressant treatment (Griffin and Mellon, 1999; Nechmad et al., 2003). The intracerebroventricular administration of allopregnanolone shows an antidepressant-like effect in the forced swim test (Khisti et al., 2000) and learned helplessness animal models of depression (Shirayama et al., 2011). The idea that allopregnanolone is one of the most important neurosteroids involved in the antidepressant effect of drugs has been confirmed by the administration of finasteride. This 5α-reductase inhibitor, decreases the conversion of progesterone to 5α-dehydroprogesterone and subsequently to allopregnanolone, and increases immobility behaviors in the forced swim test (Beckley and Finn, 2007).

Limbic brain regions are implicated in mood disorders and studies point to the nucleus accumbens as an important area for emotional control (Sheline, 2003). The nucleus accumbens has extensive input from and output to other limbic areas, such as the hippocampus (Goto and O'Donnell, 2001; Nauta et al., 1978). Because there is an inverse correlation between hippocampal allopregnanolone levels and depressive-like behavior in female rats it has been supposed that the hippocampus is implicated in the antidepressant effect of this neurosteroid (Frye and Walf, 2002, 2004). Indeed, intra-hippocampal infusion of allopregnanolone

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reduces the immobility of rats in the forced swim test (Nin et al., 2008). Furthermore, when administered into the nucleus accumbens, allopregnanolone also reduces immobility duration in rats (Molina-Hernandèz et al., 2005), leading to the conclusion that there might be more than one brain areas related to the antidepressant effects of neurosteroids or that the allopregnanolone infusion in a certain limbic area may evoke GABAergic system alteration in the hippocampus.

$GABA_A$ receptor function is modulated by GABA and also by benzodiazepines, barbiturates, convulsants and neurosteroids, including allopregnanolone (Compagnone and Mellon, 2000). After intrahippocampal allopregnanolone administration, mRNA expression of the γ2 $GABA_A$ receptor subunit increases in the hippocampus of rats during the forced swim test (Nin et al., 2008), and expression of the δ subunit increases in the CA1, CA3 and dentate gyrus regions of the hippocampus after social isolation in mice (Serra et al., 2006). The socially isolated mouse model evokes behavioral deficits that are comparable to those of depression (Pinna et al., 2003, 2004). These deficits are associated with decreases in allopregnanolone levels in several brain areas (Pinna et al., 2003, 2006) and can be normalized with antidepressant treatment (reviewed in Pinna, 2010). Different stress models in rodents increase not only corticosterone, but also depressive-like behaviors in the forced swim test that are reversed by antidepressant treatment (Rayen et al., 2011; Viana et al., 2008; Weathington et al., 2011). Grooming is an important part of rodent behavior that can be induced by exposure to a new environment. This behavior represents a complex sequence of patterns sensitive to GABAergic drugs (Barros et al., 1992; Nin et al., 2012), and grooming is a predictor of stress-like behavior (Kalueff and Tuohimaa, 2005).

The regulation of negative emotions in normal humans depends on hemispheric asymmetries: left frontal activation is related to the voluntary suppression of negative emotions, and the right frontal area is connected to spontaneous negative emotional responses (Jackson et al., 2000). Several changes in brain asymmetry for certain brain areas have been detected with neuroimaging in mood disorder patients (Soares and Mann, 1997), including patients with major depressive disorders (Lacerda et al., 2003). Patients with bipolar disorder and depression show motor, perceptual and emotional functional disturbances associated with interhemispheric asymmetries in many brain regions, reinforcing the hypothesis that an asymmetric component may be critical for mood regulation (Caligiuri et al., 2004; Jackson et al., 2003; Tranel et al., 2002). There are hemispheric asymmetries in biochemical markers in animals subjected to unpredictable tones and shocks (Orman and Stewart, 2007), which may trigger coping problems in these animals that are associated with stressful stimuli (Sullivan, 2004). In a study from our laboratory, in rats, we observed that following allopregnanolone treatment, antidepressant-like effects and higher mRNA expression of the γ2 $GABA_A$ subunit the right hippocampus compared to the left hippocampus were detected (Nin et al., 2008).

Our present study was designed to determine the antidepressant and antistress-like effects of intra-nucleus accumbens infusion of allopregnanolone in rats and to quantify the hippocampal δ and γ2 $GABA_A$ receptor subunits expression in these animals, correlating the behaviors observed with the biochemical data.

2. Methods and materials

2.1. Animals

Male Wistar rats (250–280 g; 90–110 days; $n=42$) were obtained from the Animal House of Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA). Before surgery, the animals were housed in groups of five in polypropylene cages with wood shavings as bedding. After surgery, the animals were maintained in isolated cages ($25\times35\times35$ cm in height). Food and water were available *ad libitum*, and the animals were maintained in a temperature-controlled room (22 ± 2 °C) under a light–dark cycle (lights on from 7 am–7 pm). All *in vivo* experiments followed the guidelines of the International Council for Laboratory Animal Science and were approved by the Ethical Committee for Research of UFCSPA (557/08). All efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data.

2.2. Treatments

Immediately before administration, allopregnanolone (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in a 20% (w/v) 2-hydroxypropyl-β-cyclodextrin (Fluka-Sigma-Aldrich, St. Louis, MO, USA) solution prepared in artificial cerebrospinal fluid (ACSF) (NaCl 147 mM; $CaCl_2$ 2.3 mM; KCl 4 mM; $MgCl_2$ 0.9 mM; pH 7.1–7.3) to obtain a concentration of 1.25 μg/μL, 2.25 μg/μL and 5 μg/μL. These doses showed antidepressant-like effects when infused in the hippocampus (Nin et al., 2008), and similar doses do not affect locomotion when infused in the nucleus accumbens and in the hippocampus of rats (Molina-Hernandèz et al., 2005; Rodríguez-Landa et al., 2009). The vehicle, infused in the control rats (0 μg/rat), was the 20% 2-hydroxypropyl-β-cyclodextrin ACSF solution. The animals were randomly divided into four subgroups. Allopregnanolone (1.25, 2.5, or 5 μg/rat) or vehicle were infused in each hemisphere in a volume of 0.5 μL per rat. The solutions were infused at a constant rate of 0.25 μL/min through a microperfusion pump (CMA/102. Acton; Harvard Apparatus, Holliston, MA, USA) connected to a 27-gauge needle that was introduced 0.2 mm below the end of the guide cannula. To avoid reflux, the injection needles were removed from the guide cannula 2 min after the end of the infusions. Allopregnanolone or vehicle solution was bilaterally administered three times: 24, 5 and 1 h before the test section of the behavioral tests. The same animals were used for all of the behavioral and biochemistry experiments. Each experimental group included 10–11 rats for the behavioral data and 6–9 rats for the biochemical data.

2.3. Surgery procedure

The rats were anesthetized with xylazine HCl (5 mg/kg) and ketamine HCl (100 mg/kg) i.p. and placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA). Bilateral cannulae were placed in the nucleus accumbens core (anteroposterior: +1.6 mm from bregma; lateral: ±1.8 mm from bregma; vertical: −6.6 mm from dura mater according to Paxinos and Watson, 1998). The nucleus accumbens core was chosen because it is an area related to selection and integration of the limbic inputs that acquire control over motivation, discrete cues and learning (Ito and Hayen, 2011) which are considered important in depression. Although the surgery procedure to introduce the cannulae may provoke injuries, it is minimal, since the cannulae trespasses vertically the beginning of the hippocampus, leaving most part of it intact (Paxinos and Watson, 1998). The endpoints of the cannulae were 0.2 mm under the target, and the infusion areas are presented in Fig. 1. The cannulae were fixed to the skull with two screws and dental cement. The surgeries were performed 7 ± 2 days prior to the training day component of the tests.

2.4. Grooming microstructure test

Forty-five minutes after the last treatment dose, the animals were subjected to the behavioral tests. For grooming analyses, the animals were placed inside a cylinder with a diameter of 20 cm surrounded by 30-cm high white walls and a transparent floor. A camera placed 10 cm below the apparatus floor was used to record animals' behaviors and provide a detailed record of activity. The test comprised of two sections 24 h apart: training (15 min) and test (15 min), both of which were performed between 1 and 5 pm. In both sections, each rat was placed in the center of the cylinder. Only the test section was recorded. The testing room was illuminated by a dim light (25 W), and the

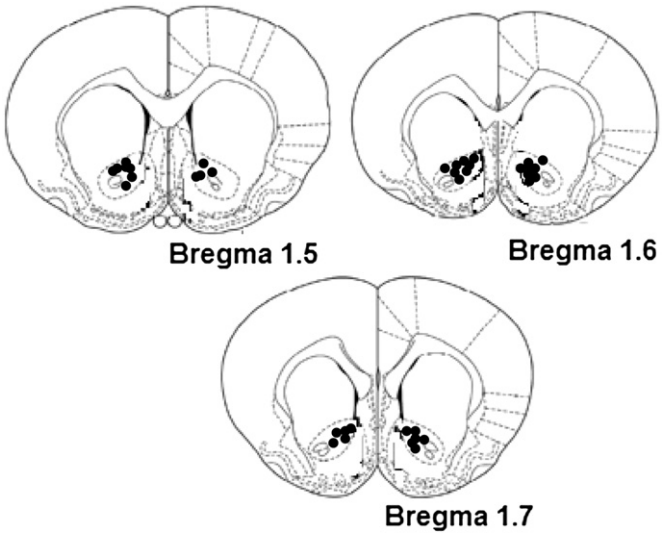

Fig. 1. A schematic representation of microinjection sites within the nucleus accumbens core in +1.5 mm; +1.6 mm and +1.7 mm from bregma.

cylinder was cleaned with ethanol (70%) after each test. A classical analysis of grooming behaviors was performed and consisted of the number of bouts (NBs) (previously called frequency of grooming), latency to start grooming, and total time of grooming (TTG) (Barros et al., 1994; Kalueff and Tuohimaa, 2005). The modified grooming microstructure analysis was measured by the grooming actions sequence: (1) paw licking, (2) snout washing, (3) head washing, (4) body grooming/scratching, (5) hind legs and genital washing, and (0) no grooming (Kalueff and Tuohimaa, 2005). An action, scored in number of actions (NA), refers to any one the behaviors numbered above, except for "0-no grooming". The quantification of the grooming action sequence was performed using an algorithm for posterior analysis (Kalueff and Tuohimaa, 2004, 2005). A bout, quantified as the number of bouts (NBs), is a single action that is interrupted by a no-grooming action (0) for at least 3 s, or a sequence of actions until they are interrupted. A complete bout (CB) consists of the following sequence of actions: 0–1–2–3–4–5–0; or a shorter one: 1–2–3–4–5. The following parameters were also determined: the number and percentage of complete bouts (NCBs) and incomplete bouts (NIB = NB − NCB), average duration of a bout (ADB), average duration of an action (ADA) and average number of transitions per bout (ANT = NA/NB). In addition, the number of correct transitions (CT) (0–1; 1–2; 2–3; 3–4; 4–5; 5–0), the number of incorrect transitions (IT, any other transitions), the total number of transitions (NT) and the percentage of correct (%CT = CT/NT) and incorrect transitions (%IT = IT/NT) were also analyzed, according to Kalueff and Tuohimaa (2005).

2.5. Forced swim test

Immediately after the grooming test, the animals were subjected to the forced swim test. The procedure followed a slightly modified Porsolt forced swim test protocol (Detke et al., 1995). All behavioral tests were performed between 12 pm and 4 pm. Briefly, the animals were individually placed in cubic glass pools (25 × 25 × 40 cm) filled with 30 cm of water at a temperature of 25(±1) °C. Although the cylindrical pool is classically used for the forced swim test, the cubic swimming pool was demonstrated to have the same sensitivity to detect the antidepressant effect of drugs and has been used as a model in our group and by other researchers (Ferigolo et al., 1998; Gomez et al., 2003; Molina-Hernandèz et al., 2005; Nin et al., 2008). Two swim sections were conducted: an initial 15 min training section followed 24 h later by a 5-min test session. Following both sessions, the rats were removed from the pools, carefully dried with towels, placed in a heated room for 15 min and later returned to their home cages.

The frequency and duration of acts and postures (climbing, swimming, diving, head-shakes, total mobility and immobility, and latency to immobility) were detailed for each animal during the analysis of the 300 s in the test day section. The behavioral analyses were described previously and the model showed both reliability and validity for detecting the antidepressant effects of noradrenergic, serotoninergic, dopaminergic and GABAergic drugs in the forced swim test (Detke et al., 1995; Ferigolo et al., 1998; Miura et al., 1996; Nin et al., 2008). All of the behavioral analyses were video-recorded and were analyzed by the same observer, who was blind to the animal group allocation. The behaviors of interest were quantified using the software program qbasic-wabehave written in BASIC (Kevin Willioma, KD Ware Computer, Boston, MA, USA).

2.6. Cannulae placement verification and structures removal

At the end of the behavioral testing, the animals were euthanized by decapitation. The brains were rapidly removed, frozen in liquid nitrogen and stored in a freezer at −80 °C. After briefly thawing, the brains were sectioned using a rat brain slicer 1.0 mm (Zivic, Pittsburgh, PA, USA) into the cannula incision area and photographed for posterior placement analysis (Samsung™–DigimaxV5), which was followed by hippocampus removal. Only animals with accurately placed cannulae were considered for neurochemical and behavioral analysis. Correct cannulae placement was verified in 42 of the 49 animals that were submitted to surgery: vehicle ($n = 11$), 1.25 μg/rat ($n = 11$), 2.5 μg/rat ($n = 10$) and 5 μg/rat ($n = 10$).

2.7. Real-time RT-PCR

The analysis of the relative gene expression of the γ2 and δ $GABA_A$ subunit receptors in both hippocampus of each hemisphere was performed using reverse transcription combined with real-time quantitative PCR (qPCR) and the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). For hippocampal gene expressions 6 to 9 samples were used, randomly chosen from the animals submitted to the behavioral protocols. Total RNA was extracted from each hemisphere of the hippocampus using the Trizol™ Isolation Reagent Kit (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. All RNA samples were resuspended in 50 μL of DEPC-treated water (Life Technologies, Carlsbad, CA, USA) and stored at −80 °C. The cDNA was synthesized from 9 μL of each RNA sample using SuperScript III (Life Technologies, Carlsbad, CA, USA). The quantification was made by spectrophotometry using a Nanodrop (Thermo Fisher Scientific, Wilmington, DE, USA). qPCR analysis was performed at least in duplicate using the StepOnePlus Real-Time PCR System (Life Technologies, Carlsbad, CA, USA). The set of δ and γ2 $GABA_A$ subunit and β-actin (endogenous control) primers was determined based on *Rattus norvegicus* data from the National Center for Biotechnology Information (Table 1). The reaction contained 50 ng of cDNA, 2xPower SYBR Green PCR Master Mix (Life Technologies, Carlsbad, CA, USA) and 0.17 μM of each primer. The genes were amplified with an initial denaturation at 95 °C for 10 min followed by 40 cycles at 95 °C for 15 s and 60 °C for 1 min and were succeeded by the melting curve stage (Table 1).

2.8. Statistical analysis

A one-way ANOVA was used to determine if there were effects of the different doses of allopregnanolone on the behavior of rats submitted to the forced swim and microstructure grooming tests. A two-way ANOVA was used to compare the right and left hemisphere mRNA expression and allopregnanolone treatments. Tukey's post-hoc test was used when appropriate. Pearson's correlation was used to verify if there was any association between behaviors in the forced

Table 1
Characteristics of primers used for amplification.

Primer	Sequence	Size (bp)	TM	GenBank
b-actin (sense)	5′-TATGCCAACAC AGTGCTGTCTGG-3′	205	82.2 °C	NM_031144.2
b-actin (antisense)	5′-TACTCCTGCTTG CTGATCCACAT-3′			
δ (sense)	5′-AGCAGTGCCT GCCAGAGTAT-3′	563	84.4 °C	NM_017289.1
δ (antisense)	5′-CATGTAAAGC CGTCATGTGG-3′			
γ2 (sense)	5′-GACGATGACC ACTCTCAGCA-3′	402	80.1 °C	NM_177408.5
γ2 (antisense)	5′-ACAGTCCTTG CCATCCAAAC-3′			

TM, melting temperature; bp, base pairs; δ or γ2, delta or gamma-2 GABAA receptor subunit, respectively; GenBank and accession numbers are available at http://www.ncbi.nlm.nih.gov/.

swim test or grooming test and gene expression data. All data are presented as the mean ± standard error of the mean (S.E.M.). Differences were considered significant at $P<0.05$.

3. Results

3.1. Effects of allopregnanolone on forced swim test behavior (see Fig. 2)

Intra-nucleus accumbens infusion of allopregnanolone decreased the immobility time [$F_{(3,38)}=3.36$, $P=0.029$; Fig. 2A] of rats submitted to the forced-swimming which demonstrates its antidepressant-like effect. Allopregnanolone-treated animals at the 5 μg/rat dose spent 36% less time in immobile-like behaviors than control rats (control: 202.0 ± 48.0; ALLO 5 μg/rat: 129.1 ± 63.6; $P=0.019$). This decrease of immobility is concomitant to enhanced climbing behavior [$F_{(3,38)}=4.26$, $P=0.011$; Fig. 2B] at the same dose ($P=0.007$), without any differences in the swimming time [$F_{(3,38)}=0.69$, $P=0.561$]. Also, the

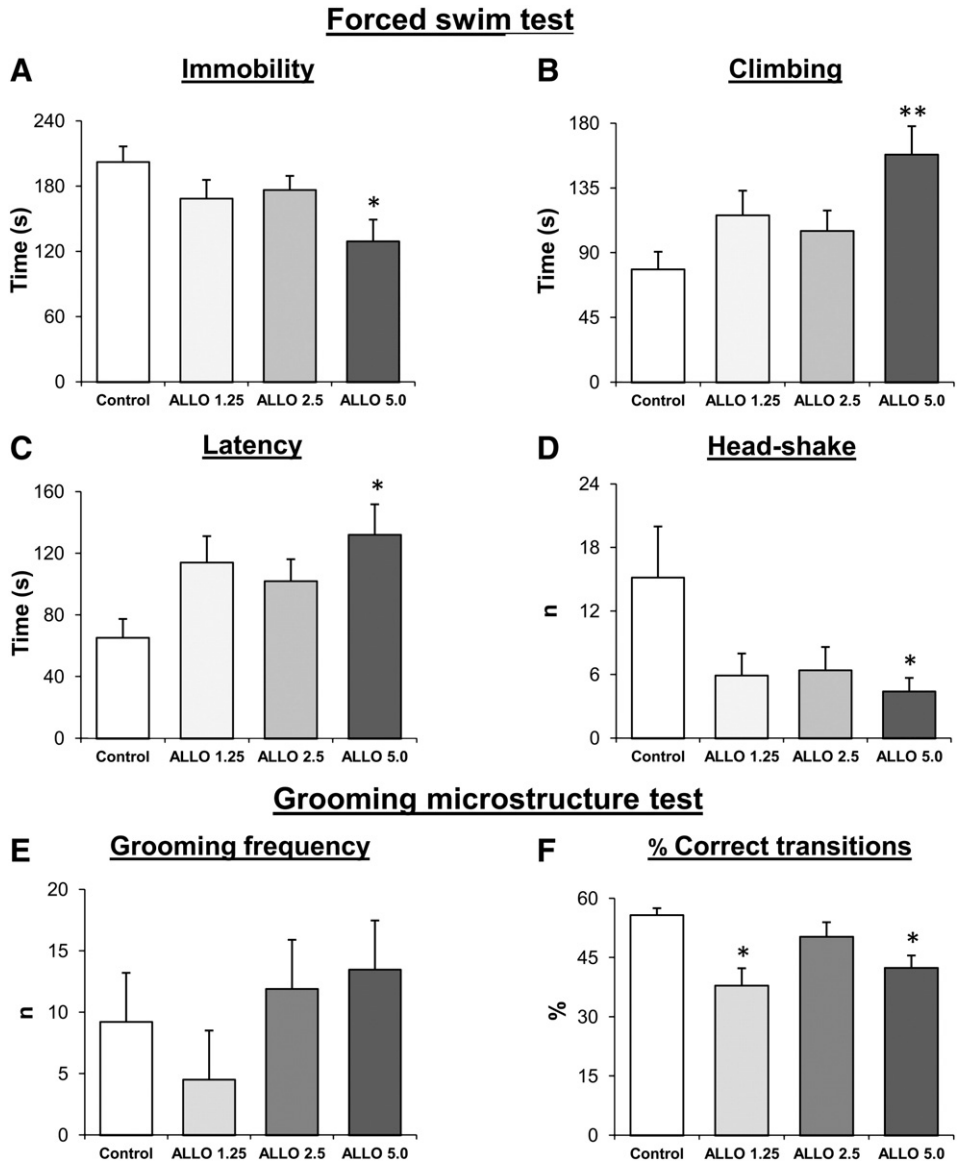

Fig. 2. Effect of allopregnanolone (ALLO) infusion in the nucleus accumbens on behaviors in the forced swim and grooming microstructure tests. ALLO was bilaterally infused at doses of 1.25, 2.5 or 5 μg/rat, 24, 5 and 1 h before the behavioral tests. Forced swim test analysis: immobility (A), climbing (B), latency to start immobility (C) and head-shake (D). Grooming microstructure test: grooming frequency (E) and percent of correct transitions (F). Each bar represents the mean ± S.E.M. The comparisons were made using a one-way ANOVA and Tukey's test when appropriate. $^{*}P<0.05$, $^{**}P<0.01$ compared with the control group.

latency to start immobility was increased in the same proportion [$F_{(3,38)}=3.20$, $P=0.039$; Fig. 2C] with the allopregnanolone 5 μg/rat group differing from the control group ($P=0.035$). Another behavior that was decreased by the 5 μg/rat allopregnanolone treatment was frequency of head-shakes [$F_{(3,38)}=3.06$, $P=0.040$; Fig. 2D] compared with the control group ($P=0.035$). There were no statistical differences in behaviors after intra-nucleus accumbens administration of allopregnanolone at doses of 1.25 or 2.5 μg/rat.

3.2. Effect of allopregnanolone on grooming microstructure test behavior

Allopregnanolone infusion did not alter classical grooming measurements, such as the total time of grooming, frequency of grooming (Fig. 2E) or latency to start grooming. Nevertheless, allopregnanolone infusion changed grooming microstructure parameters. In fact, the intra-nucleus accumbens infusion of allopregnanolone decreased the percentage of correct transitions [$F_{(3,29)}=3.82$, $P=0.023$; Fig. 2F] when the allopregnanolone 1.25 μg/rat ($P=0.044$) and 5 μg/rat groups ($P=0.039$) were compared with the control group. In addition, the percentage of incorrect transitions was changed because it is complementary to the percentage of correct transitions. No other statistical differences were observed in the microstructure grooming test after allopregnanolone treatment.

3.3. $GABA_A$ subunit mRNA expression after allopregnanolone infusion

The main purpose of this experiment was to determine if allopregnanolone infused in the nucleus accumbens would alter the $GABA_A$ subunit expression in the hippocampus, which could explain the decrease in forced swim test immobility. Allopregnanolone infusion in the nucleus accumbens altered mRNA expression of the δ $GABA_A$ subunit in the hippocampus, depending on the hemisphere analyzed [$F_{(3,56)\text{interaction}}=3.27$, $P=0.028$; Fig. 3A]. Treatment with 5 μg/rat allopregnanolone increased δ expression compared with the control group, only in the right hemisphere ($\text{control}_{\text{right}}$: 1.21 ± 0.31; ALLO 5 $\mu g/\text{rat}_{\text{right}}$: 5.99 ± 0.95; $P<0.001$), which was also higher than the left hippocampal hemisphere at the same dose (ALLO 5 $\mu g/\text{rat}_{\text{left}}$: 1.02 ± 0.23; $P<0.001$). Considering the other $GABA_A$ receptor subunit tested, γ2, the profile was almost identical, with a significant interaction being observed between the treatment and the hemisphere [$F_{(3,56)\text{interaction}}=3.52$, $P=0.023$; Fig. 3B]. Rats treated with 5 μg/rat allopregnanolone showed an increase in γ2 subunit expression in the right hemisphere compared with the left hemisphere (ALLO 5 $\mu g/\text{rat}_{\text{left}}$: 0.60 ± 0.14; ALLO 5 $\mu g/\text{rat}_{\text{right}}$: 2.3 ± 0.52; $P<0.001$) and with the control treatment ($\text{control}_{\text{right}}$: 1.17 ± 0.23; $P=0.029$). There was no significant correlation between the major parameters of the forced swim and grooming microstructure tests and the biochemical data.

4. Discussion

In this study, allopregnanolone produced an antidepressant-like effect in rats after intra-nucleus accumbens core infusion (5 μg/rat), as evaluated by the forced swim test. The region-specific effect of allopregnanolone has been described to be dependent on the depressive animal model used (Shirayama et al., 2011; Uzunova et al., 2004). Although our study describes a specific coordinate targeting the core of the nucleus accumbens, we may assume that allopregnanolone solution diffuses to adjacent shell region. A whole brain effect, as observed with intracerebroventricular allopregnanolone treatment, induces an antidepressant-like state in the learned helplessness model (Shirayama et al., 2011). More specifically, some areas seem to play a major role in this effect, such as the hippocampus, in which the administration of allopregnanolone decreases depressive-like behavior, as evaluated by the forced swim test (Nin et al., 2008; Rodríguez-Landa et al., 2009). Curiously, there is an antidepressant-like effect of allopregnanolone when infused in the CA3 region but not in the dentate gyrus, as evaluated by the learned helplessness rat model (Shirayama et al., 2011). The antidepressant-like effect of allopregnanolone infused in the nucleus accumbens shown in this study was not observed in the learned helplessness rat model when it was tested at smaller doses (1.0 and 0.1 μg/rat) than in this study (Shirayama et al., 2011). In addition, there was no antidepressant-like effect of allopregnanolone infusion in the dorsal region of the prefrontal cortex or the caudatum putamen nucleus in the forced swim test at the same doses used in the present study (unpublished results).

Paralleling these results, it has been observed that in the olfactory bulbectomized rat animal model of depression, there is a decrease in allopregnanolone levels in the amygdala, frontal cortex and hippocampus (Uzunova et al., 2003). The nucleus accumbens has extensive input from and output to different limbic areas, such as the hippocampus (Goto and O'Donnell, 2001; Nauta et al., 1978), and its main neuronal cell type is the medium spiny neuron, which is a GABAergic neuron. Nucleus accumbens core and shell receive different afferent projections (Groenewegen et al., 1999), and some brain regions project to both the nucleus accumbens core and shell (Voorn et al., 2004; Fields et al., 2007; Humphries and Prescott, 2010). Consequently, nucleus accumbens core and shell neurons could encode similar information but have different effects on behavior depending on the downstream regions they innervate. For instance, the transection or stimulation of fibers which carry hippocampal afferent information to the nucleus accumbens, result in disappearance or induction, respectively, of the more depolarized state in nucleus accumbens neurons (O'Donnell and

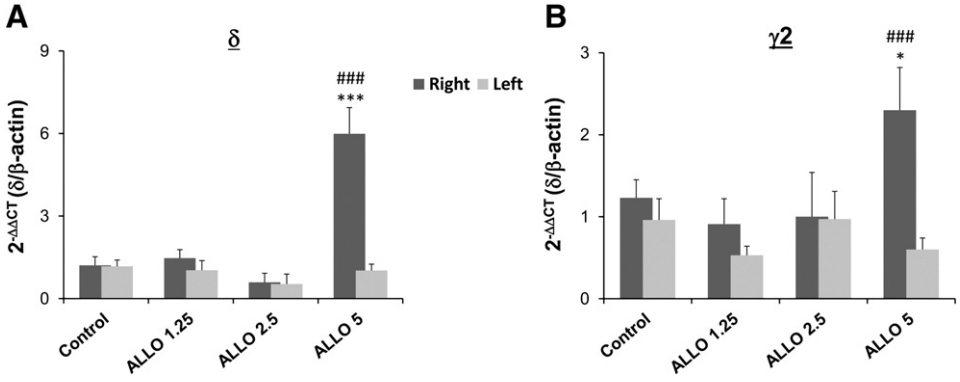

Fig. 3. Effect of allopregnanolone (ALLO) infusion in the nucleus accumbens on $GABA_A$ receptor expression in the hippocampus. (A) δ and (B) γ2 mRNA $GABA_A$ subunit expression according to real-time RT-PCR. The treatments with ALLO 1.25, 2.5 or 5 μg/rat were bilaterally measured in the left and right hemispheres. Each bar represents the mean ± S.E.M. The comparisons were made using a two-way ANOVA and Tukey's test when appropriate. $^{*}P<0.05$, $^{***}P<0.001$ compared with the control group in the same hemisphere. $^{###}P<0.001$ compared with the same treatment group in the other hemisphere.

Grace, 1995). In addition to previous information, Goto and O'Donnell (2001) described electrical ensembles between hippocampus and nucleus accumbens neurons. The role of the hippocampus in the antidepressant-like effects of allopregnanolone was confirmed by a previous study from our group, when the hippocampal infusion of allopregnanolone decreased the depressive-like state in rats (Nin et al., 2008). Therefore, beyond the idea that the antidepressant effect may be region-specific, it is also plausible that increases in allopregnanolone through an endogenous mechanism or by exogenous infusion in one specific brain area could project its activity to some other functionally connected brain area, as was observed in this study.

Climbing behavior during the forced swim test is used as a marker for the norepinephrine activity in the central nervous system altered by antidepressants (Detke, et al., 1995). Interestingly, a climbing increase was previously observed after allopregnanolone treatment in rats, with no change in swimming behavior (Molina-Hernandèz et al., 2005; Nin et al., 2008). Until now, there are no descriptions of differential behavioral changes in the forced swim test induced by GABAergic drugs that have antidepressant-like effects. The anti-immobility effects of several GABAergic agents (Khisti et al., 2000; Skirzewski et al., 2011) or agents that change neurosteroid levels in the brain (Espallergues et al., 2012) have been reported without measuring other behaviors usually observed during the test. Therefore, we may speculate that this increase in climbing would also be observed with GABAergic drugs. In contrast to climbing behavior, there was a decrease in the head-shake behavior when allopregnanolone was infused in the nucleus accumbens; however, this behavior was not observed when allopregnanolone was infused in the hippocampus (Nin et al., 2008). Although head-shake behavior is known to be a typical serotoninergic-associated effect (Benekareddy et al., 2010; Willins and Meltzer, 1997), changes in this behavior could also be due to activity in the GABAergic system (Vargas et al., 1996, 1998). This pattern of effects was also observed after treatment with non specific antidepressants, like imipramin (Barros and Ferigolo, 1998; Ferigolo et al., 1998).

Stressful situations, as occur in the socially isolated mouse model, evoke behavioral deficits that are analogous to depression (Pinna, 2010). Interestingly, allopregnanolone levels are also decreased in the corticolimbic structures of this socially isolated mouse model (Agis-Balboa et al., 2007; Pinna et al., 2003, 2006). Chronic treatment with corticosterone, mimicking stressed animal levels, alters GABAergic function in the hippocampus of rats (Orchinik et al., 1995). Moreover, stress induced by the forced swim test produces fluctuations in the GABA levels of animals (Gomez et al., 2003; Skirzewski et al., 2011), and certain antidepressants, such as paroxetine, can decrease the levels of corticosterone (Linthorst and Reul, 2008). Stress induction, such as the exposure to a new environment, induces grooming behavior, which can be observed early after moving the animal from its cage to a new arena or when the animal is exposed to other stressors (Bindra and Spinner, 1958; Kalueff and Tuohimaa, 2005). Moreover, the grooming microstructure test seems to be a more sensitive tool to detect stress-like situations in rodents than the classical grooming parameters (Kalueff and Tuohimaa, 2004, 2005). In the present study, it was observed that allopregnanolone (1.25 and 5 μg/rat) treatment reduced the percentage of correct transitions, which is described as a predictor of stress-like behavior (Kalueff and Tuohimaa, 2005). First, we hypothesized that allopregnanolone would increase the percentage of correct transitions because it is a GABAergic drug with known anxiolytic effects (Akwa et al., 1999; Bitran et al., 2000); however, we observed an antagonistic effect, with the lowest and highest doses decreasing that parameter. It has been suggested that several $GABA_A$ modulators have biphasic effects, with low doses increasing negative effects, such as anxiety, and higher doses decreasing them. This biphasic effect is observed after allopregnanolone treatment in aggression (Miczek et al., 1997) and avoidance/anxiety behavioral tests (Beauchamp et al., 2000). Our results may agree with part of this biphasic effect, because the lowest dose decreased the percentage of correct transitions, which is a predictor of an anxiogenic effect.

The $GABA_A$ receptor is composed of five subunits, and its function depends on combinations of these subunits (α1-6, β1-3, γ1-3, δ, ε, π, o and ρ1-3) (Hedblom and Kirkness, 1997; Hevers and Luddens, 1998; Mehta and Ticku, 1999). It was reported that allopregnanolone increases the expression of γ2 subunit mRNA in the hippocampus after intrahippocampal infusion (Nin et al., 2008). Also, it is known that mice that are heterozygous for the γ2 subunit of $GABA_A$Rs ($\gamma2^{+/}$) exhibit a modest functional deficit in $GABA_A$-R (Crestani et al., 1999), accompanied by depressant-like symptoms, observed in the forced swim and in the tail suspension tests (Shen et al., 2010). In the same direction, γ2 L $GABA_A$ receptor subunits increase during pregnancy with concomitantly higher levels of circulating allopregnanolone in the cerebral cortex and hippocampus (Concas et al., 1999). In the present study, we also observed an increase in γ2 expression in the right hippocampus at the same dose that improved behavioral deficits. The δ subunit of the $GABA_A$ receptor has been described to play a pivotal role in neurosteroid modulation, and its absence prevents inhibitory synaptic currents in certain brain cells (Vicini et al., 2002). After 48 h of allopregnanolone treatment, there is an increase in the protein expression of the δ $GABA_A$ subunit in the hippocampus of female rats (Shen et al., 2005). The present study also showed that there was an increase in the δ $GABA_A$ subunit mRNA expression in the right hippocampal hemisphere of male rats after the allopregnanolone treatment dose that reversed depressant-like behaviors. In the present case, the biochemical analyses were performed in both brain hemispheres, and the increase in δ and γ2 subunit expression after allopregnanolone treatment was observed in the right hemisphere. Compared with the study in females (Shen et al., 2005), in the present study, when the mRNA data from both hemispheres is combined, there is still an overall increase in expression of the δ and γ2 subunits. Previous studies from our group have shown that γ2 $GABA_A$ receptor subunit gene expression increases in the right hemisphere compared with the left one after allopregnanolone treatment (Nin et al., 2008). Asymmetrical changes in brain biochemistry may also be present in mood disorders in humans (Soares and Mann, 1997). Mood disorders, such as bipolar disorder and depression, are associated with right hemisphere dysfunction (Caligiuri et al., 2004; Tranel et al., 2002), and hemispheric asymmetry has also been observed in animal behavioral studies (Orman and Stewart, 2007; Sullivan, 2004). In addition, other receptors, such as the *N*-methyl-D-aspartate receptor, and some of its subunits, have higher right hemisphere subunit density in the mouse hippocampus (Kawakami et al., 2003; Wu et al., 2005). This phenomenon is not observed for $GABA_A$ receptors, which exhibit symmetrical expression in untreated animals. Moreover, the asymmetry observed here is only present in the animals in which the depressant-like state was reversed, suggesting a drug induction asymmetry and not a disorder induction asymmetry. All of this information may imply that the allopregnanolone antidepressant effect is not only related to the altered abundance of certain receptors in different areas but also to the laterality of this change. The use of neurosteroids in the treatment of mood disorders area currently under investigation. Some synthetic neurosteroids has been tested with respect to their therapeutic potential as ganaxolone, alphaxalone and minaxolone (reviewed in Schüle et al., 2011). Thus, studies exploring the mechanism of action of allopregnanolone are important tool to discovery of new antidepressants.

5. Conclusion

This study provides evidence for the antidepressant-like effect of intra-nucleus accumbens core infusion with allopregnanolone, confirming the participation of neurosteroids in the pathophysiology of depression. The grooming microstructure analysis suggests that

there may be a paradoxical biphasic effect at the lowest allopregnanolone dose. Both δ and γ2 $GABA_A$ subunit expression increases in the hippocampus may have a role in the mechanism of the allopregnanolone antidepressant effect, providing information not only regarding the directly infused region but also about changes in other brain areas of the limbic system. Our findings point to asymmetrical brain changes in $GABA_A$ receptor mRNA expression in animals treated with allopregnanolone. Further investigation should attempt to detect which other brain areas are involved in this GABAergic mechanism of action of allopregnanolone and whether this asymmetry persists after chronic treatment.

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References

Agis-Balboa RC, Pinna G, Pibiri F, Kadriu B, Costa E, Guidotti A. Down-regulation of neurosteroid biosynthesis in corticolimbic circuits mediates social isolation-induced behavior in mice. Proc Natl Acad Sci U S A 2007;104:18,736–41.

Akwa Y, Purdy RH, Koob GF, Britton KT. The amygdala mediates the anxiolytic-like effect of the neurosteroid allopregnanolone in rat. Behav Brain Res 1999;106: 119–25.

Barros HM, Ferigolo M. Ethopharmacology of imipramine in the forced-swimming test: gender differences. Neurosci Biobehav Rev 1998;23:279–86.

Barros HM, Tannhauser SL, Tannhauser MA, Tannhauser M. Effect of sodium valproate on the open-field behavior of rats. Braz J Med Biol Res 1992;25:281–7.

Barros HM, Tannhauser SL, Tannhauser MA, Tannhauser M. The effects of GABAergic drugs on grooming behaviour in the open field. Pharmacol Toxicol 1994;74:339–44.

Beauchamp MH, Ormerod BK, Jhamandas K, Boegman RJ, Beninger RJ. Neurosteroids and reward: allopregnanolone produces a conditioned place aversion in rats. Pharmacol Biochem Behav 2000;67:29–35.

Beckley EH, Finn DA. Inhibition of progesterone metabolism mimics the effect of progesterone withdrawal on forced swim test immobility. Pharmacol Biochem Behav 2007;87:412–9.

Benekareddy M, Goodfellow NM, Lambe EK, Vaidya VA. Enhanced function of prefrontal serotonin 5-HT(2) receptors in a rat model of psychiatric vulnerability. J Neurosci 2010;30:12,138–50.

Bianchi M, Baulieu EE. 3β-Methoxy-pregnenolone (MAP4343) as an innovative therapeutic approach for depressive disorders. Proc Natl Acad Sci U S A 2012;109:1713–8.

Bindra D, Spinner N. Response to different degrees of novelty: the incidence of various activities. J Exp Anal Behav 1958;1:341–50.

Bitran D, Foley M, Audette D, Leslie N, Frye CA. Activation of peripheral mitochondrial benzodiazepine receptors in the hippocampus stimulates allopregnanolone synthesis and produces anxiolytic-like effects in the rat. Psychopharmacology (Berl) 2000;151:64–71.

Caligiuri MP, Brown GG, Meloy MJ, Eyler LT, Kindermann SS, Eberson S, et al. A functional magnetic resonance imaging study of cortical asymmetry in bipolar disorder. Bipolar Disord 2004;6:183–96.

Compagnone NA, Mellon SH. Neurosteroids: biosynthesis and function of these novel neuromodulators. Front Neuroendocrinol 2000;21:1-56.

Concas A, Follesa P, Barbaccia ML, Purdy RH, Biggio G. Physiological modulation of GABA(A) receptor plasticity by progesterone metabolites. Eur J Pharmacol 1999;375:225–35.

Crestani F, Lorez M, Baer K, Essrich C, Benke D, Laurent JP, et al. Decreased GABAA-receptor clustering results in enhanced anxiety and a bias for threat cues. Nat Neurosci 1999;2:833–9.

Detke MJ, Rickels M, Lucki I. Active behaviors in the rat forced swimming test differentially produced by serotonergic and noradrenergic antidepressants. Psychopharmacology 1995;121:66–72.

Espallergues J, Mamiya T, Vallée M, Koseki T, Nabeshima T, Temsamani J, et al. The antidepressant-like effects of the 3β-hydroxysteroid dehydrogenase inhibitor trilostane in mice is related to changes in neuroactive steroid and monoamine levels. Neuropharmacology 2012;62:492–502.

Ferigolo M, Barros HM, Marquardt AR, Tannhauser M. Comparison of behavioral effects of moclobemide and deprenyl during forced swimming. Pharmacol Biochem Behav 1998;60:431–7.

Fields HL, Hjelmstad GO, Margolis EB, Nicola SM. Ventral tegmental area neurons in learned appetitive behavior and positive reinforcement. Annu Rev Neurosci 2007;30: 289–316.

Frye CA, Walf AA. Changes in progesterone metabolites in the hippocampus can modulate open field and forced swim test behavior of proestrous rats. Horm Behav 2002;41:306–15.

Frye CA, Walf AA. Hippocampal 3alpha,5alpha-THP may alter depressive behavior of pregnant and lactating rats. Pharmacol Biochem Behav 2004;78:531–40.

Gomez R, Vargas CR, Wajner M, Barros HM. Lower *in vivo* brain extracellular GABA concentration in diabetic rats during forced swimming. Brain Res 2003;968:281–4.

Goto Y, O'Donnell P. Synchronous activity in the hippocampus and nucleus accumbens *in vivo*. J Neurosci 2001;21:RC131.

Griffin LD, Mellon SH. Selective serotonin reuptake inhibitors directly alter activity of neurosteroidogenic enzymes. Proc Natl Acad Sci U S A 1999;96:13,512–7.

Groenewegen HJ, Wright CI, Beijer AV, Voorn P. Convergence and segregation of ventral striatal inputs and outputs. Ann N Y Acad Sci 1999;877:49–63.

Hedblom E, Kirkness EF. A novel class of GABAA receptor subunit in tissues of the reproductive system. J Biol Chem 1997;272:15,346–50.

Hevers W, Luddens H. The diversity of GABAA receptors. Pharmacological and electrophysiological properties of GABAA channel subtypes. Mol Neurobiol 1998;18:35–86.

Humphries MD, Prescott TJ. The ventral basal ganglia, a selection mechanism at the crossroads of space, strategy, and reward. Prog Neurobiol 2010;90:385–417.

Ito R, Hayen A. Opposing roles of nucleus accumbens core and shell dopamine in the modulation of limbic information processing. J Neurosci 2011;31:6001–7.

Jackson DC, Malmstadt JR, Larson CL, Davidson RJ. Suppression and enhancement of emotional responses to unpleasant pictures. Psychophysiology 2000;37:515–22.

Jackson DC, Mueller CJ, Dolski I, Dalton KM, Nitschke JB, Urry HL, et al. Now you feel it, now you don't: frontal brain electrical asymmetry and individual differences in emotion regulation. Psychol Sci 2003;14:612–7.

Kalueff AV, Tuohimaa P. Grooming analysis algorithm for neurobehavioural stress research. Brain Res Brain Res Protoc 2004;13:151–8.

Kalueff AV, Tuohimaa P. The grooming analysis algorithm discriminates between different levels of anxiety in rats: potential utility for neurobehavioural stress research. J Neurosci Methods 2005;143:169–77.

Kawakami R, Shinohara Y, Kato Y, Sugiyama H, Shigemoto R, Ito I. Asymmetrical allocation of NMDA receptor epsilon2 subunits in hippocampal circuitry. Science 2003;300: 990–4.

Khisti RT, Chopde CT, Jain SP. Antidepressant-like effect of the neurosteroid 3alpha-hydroxy-5alpha-pregnan-20-one in mice forced swim test. Pharmacol Biochem Behav 2000;67:137–43.

Lacerda AL, Nicoletti MA, Brambilla P, Sassi RB, Mallinger AG, Frank E, et al. Anatomical MRI study of basal ganglia in major depressive disorder. Psychiatry Res 2003;124: 129–40.

Linthorst AC, Reul JM. Stress and the brain: solving the puzzle using microdialysis. Pharmacol Biochem Behav 2008;90:163–73.

Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(−Delta Delta C(T)) method. Methods 2001;25:402–8.

Mehta AK, Ticku MK. An update on GABAA receptors. Brain Res Brain Res Rev 1999;29: 196–217.

Miczek KA, DeBold JF, van Erp AM, Tornatzky W. Alcohol, GABAA-benzodiazepine receptor complex, and aggression. Recent Dev Alcohol 1997;13:139–71.

Miura H, Naoi M, Nakahara D, Ohta T, Nagatsu T. Effects of moclobemide on forced-swimming stress and brain monoamine levels in mice. Pharmacol Biochem Behav 1996;53:469–75.

Molina-Hernandèz M, Tellez-Alcantara NP, Garcia JP, Lopez JI, Jaramillo MT. Antidepressant-like actions of intra-accumbens infusions of allopregnanolone in ovariectomized Wistar rats. Pharmacol Biochem Behav 2005;80:401–9.

Nauta WJ, Smith GP, Faull RL, Domesick VB. Efferent connections and nigral afferents of the nucleus accumbens septi in the rat. Neuroscience 1978;3:385–401.

Nechmad A, Maayan R, Spivak B, Ramadan E, Poyurovsky M, Weizman A. Brain neurosteroid changes after paroxetine administration in mice. Eur Neuropsychopharmacol 2003;13: 327–32.

Nin MS, Salles FB, Azeredo LA, Frazon AP, Gomez R, Barros HM. Antidepressant effect and changes of GABAA receptor gamma2 subunit mRNA after hippocampal administration of allopregnanolone in rats. J Psychopharmacol 2008;22:477–85.

Nin MS, Couto-Pereira NS, Souza MF, Azeredo LA, Ferri MK, Dalprá WL, et al. Anxiolytic effect of clonazepam in female rats: grooming microstructure and elevated plus maze tests. Eur J Pharmacol 2012;684:95-101.

Orchinik M, Weiland NG, McEwen BS. Chronic exposure to stress levels of corticosterone alters GABAA receptor subunit mRNA levels in rat hippocampus. Brain Res Mol Brain Res 1995;34:29–37.

Orman R, Stewart M. Hemispheric differences in protein kinase C betaII levels in the rat amygdala: baseline asymmetry and lateralized changes associated with cue and context in a classical fear conditioning paradigm. Neuroscience 2007;144:797–807.

Paul SM, Purdy RH. Neuroactive steroids. FASEB J 1992;6:2311–22.

Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 4th ed. San Diego: Academic Press; 1998.

Pinna G. In a mouse model relevant for post-traumatic stress disorder, selective brain steroidogenic stimulants (SBSS) improve behavioral deficits by normalizing allopregnanolone biosynthesis. Behav Pharmacol 2010;21:438–50.

Pinna G, Dong E, Matsumoto K, Costa E, Guidotti A. In socially isolated mice, the reversal of brain allopregnanolone down-regulation mediates the anti-aggressive action of fluoxetine. Proc Natl Acad Sci U S A 2003;100:2035–40.

Pinna G, Costa E, Guidotti A. Fluoxetine and norfluoxetine stereospecifically facilitate pentobarbital sedation by increasing neurosteroids. Proc Natl Acad Sci U S A 2004;101: 6222–5.

Pinna G, Costa E, Guidotti A. Fluoxetine and norfluoxetine stereospecifically and selectively increase brain neurosteroid content at doses that are inactive on 5-HT reuptake. Psychopharmacology 2006;186:362–72.

Rayen I, van den Hove DL, Prickaerts J, Steinbusch HW, Pawluski JL. Fluoxetine during development reverses the effects of prenatal stress on depressive-like behavior and hippocampal neurogenesis in adolescence. PLoS One 2011;6:e24003.
Rodríguez-Landa JF, Contreras CM, García-Ríos RI. Allopregnanolone microinjected into the lateral septum or dorsal hippocampus reduces immobility in the forced swim test: participation of the GABAA receptor. Behav Pharmacol 2009;20:614–22.
Romeo E, Strohle A, Spalletta G, di Michele F, Hermann B, Holsboer F, et al. Effects of antidepressant treatment on neuroactive steroids in major depression. Am J Psychiatry 1998;155:910–3.
Schüle C, Eser D, Baghai TC, Nothdurfter C, Kessler JS, Rupprecht R. Neuroactive steroids in affective disorders: target for novel antidepressant or anxiolytic drugs? Neuroscience 2011;191:55–77.
Serra M, Mostallino MC, Talani G, Pisu MG, Carta M, Mura ML, et al. Social isolation-induced increase in alpha and delta subunit gene expression is associated with a greater efficacy of ethanol on steroidogenesis and GABA receptor function. J Neurochem 2006;98:122–33.
Sheline YI. Neuroimaging studies of mood disorder effects on the brain. Biol Psychiatry 2003;54:338–52.
Shen H, Gong QH, Yuan M, Smith SS. Short-term steroid treatment increases delta GABAA receptor subunit expression in rat CA1 hippocampus: pharmacological and behavioral effects. Neuropharmacology 2005;49:573–86.
Shen Q, Lal R, Luellen BA, Earnheart JC, Andrews AM, Luscher B. gamma-Aminobutyric acid-type A receptor deficits cause hypothalamic–pituitary–adrenal axis hyperactivity and antidepressant drug sensitivity reminiscent of melancholic forms of depression. Biol Psychiatry 2010;68:512–20.
Shirayama Y, Muneoka K, Fukumoto M, Tadokoro S, Fukami G, Hashimoto K, et al. Infusions of allopregnanolone into the hippocampus and amygdala, but not into the nucleus accumbens and medial prefrontal cortex, produce antidepressant effects on the learned helplessness rats. Hippocampus 2011;21:1105–13.
Skirzewski M, López W, Mosquera E, Betancourt L, Catlow B, Chiurillo M, et al. Enhanced GABAergic tone in the ventral pallidum: memory of unpleasant experiences? Neuroscience 2011;196:131–46.
Soares JC, Mann JJ. The functional neuroanatomy of mood disorders. J Psychiatr Res 1997;31:393–432.
Strohle A, Romeo E, Hermann B, Pasini A, Spalletta G, di Michele F, et al. Concentrations of 3 alpha-reduced neuroactive steroids and their precursors in plasma of patients with major depression and after clinical recovery. Biol Psychiatry 1999;45:274–7.
Sullivan RM. Hemispheric asymmetry in stress processing in rat prefrontal cortex and the role of mesocortical dopamine. Stress 2004;7:131–43.
Tranel D, Bechara A, Denburg NL. Asymmetric functional roles of right and left ventromedial prefrontal cortices in social conduct, decision-making and emotional processing. Cortex 2002;38:589–612.
Uzunova V, Sheline Y, Davis JM, Rasmusson A, Uzunov DP, Costa E, et al. Increase in the cerebrospinal fluid content of neurosteroids in patients with unipolar major depression who are receiving fluoxetine or fluvoxamine. Proc Natl Acad Sci U S A 1998;95:3239–44.
Uzunova V, Ceci M, Kohler C, Uzunov DP, Wrynn AS. Region-specific dysregulation of allopregnanolone brain content in the olfactory bulbectomized rat model of depression. Brain Res 2003;976:1–8.
Uzunova V, Wrynn AS, Kinnunen A, Ceci M, Kohler C, Uzunov DP. Chronic antidepressants reverse cerebrocortical allopregnanolone decline in the olfactory-bulbectomized rat. Eur J Pharmacol 2004;486:31–4.
Vargas C, Tannhauser M, Tannhauser SL, Barros HM. Lithium and valproate combined administration: acute behavioural effects and drug plasma levels. Pharmacol Toxicol 1996;79:87–91.
Vargas C, Tannhauser M, Barros HM. Dissimilar effects of lithium and valproic acid on GABA and glutamine concentrations in rat cerebrospinal fluid. Gen Pharmacol 1998;30:601–4.
Viana A, Rates S, Naudin B, Janin F, Costentin J, do Rego JC. Effects of acute or 3-day treatments of *Hypericum caprifoliatum* Cham. & Schltdt. (Guttiferae) extract or of two established antidepressants on basal and stress-induced increase in serum and brain corticosterone levels. J Psychopharmacol 2008;22:681–90.
Vicini S, Losi G, Homanics GE. GABA(A) receptor delta subunit deletion prevents neurosteroid modulation of inhibitory synaptic currents in cerebellar neurons. Neuropharmacology 2002;43:646–50.
Voorn P, Vanderschuren LJ, Groenewegen HJ, Robbins TW, Pennartz CM. Putting a spin on the dorsal–ventral divide of the striatum. Trends Neurosci 2004;27:468–74.
Weathington JM, Arnold AR, Cooke BM. Juvenile social subjugation induces a sex-specific pattern of anxiety and depression-like behaviors in adult rats. Horm Behav 2011;61:91–9.
Willins DL, Meltzer HY. Direct injection of 5-HT2A receptor agonists into the medial prefrontal cortex produces a head-twitch response in rats. J Pharmacol Exp Ther 1997;282:699–706.
Wu Y, Kawakami R, Shinohara Y, Fukaya M, Sakimura K, Mishina M, et al. Target-cell-specific left–right asymmetry of NMDA receptor content in schaffer collateral synapses in epsilon1/NR2A knock-out mice. J Neurosci 2005;25:9213–26.